Profiling bacterial kinase activity using a genetic circuit

van der Helm, Eric; Bech, Rasmus; Lehning, Christina Eva; Vazquez-Uribe, Ruben; Sommer, Morten Otto Alexander

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Phosphorylation is a post-translational modification that regulates the activity of several key proteins in bacteria and eukaryotes. Accordingly, a variety of tools has been developed to measure kinase activity. To couple phosphorylation to an in vivo fluorescent readout we used the *Bacillus subtilis* kinase PtkA, transmembrane activator TkmA and the repressor FatR to construct a genetic circuit in *E. coli*. By tuning the repressor and kinase expression level at the same time, we were able to show a 4.3-fold increase in signal upon kinase induction. We furthermore validated that the previously reported FatR Y45E mutation\(^1\) attenuates operator repression. This genetic circuit provides a starting point for computational protein design and a metagenomic library-screening tool.

### 1. Design: sensing phosphorylation

**Figure 1a:** General concept of kinase activity measurement through GFP fluorescence. The repressor binds the operator site upstream of *gfp* thereby preventing transcription. The kinase phosphorylates the repressor, which attenuates binding of the repressor to the operator site, leading to transcription of *gfp* and thus fluorescence.

**Figure 1b:** An abstraction of the genetic circuit where the first inverter gate represents the phosphorylation of FatR by PtkA-TkmA on the post-translational level. The second inverter gate represents the transcriptional regulation by the binding of FatR to the operator upstream of *gfp*.

### 2. Build: circuit on two plasmids

**Figure 2:** Outline of the implementation with FatR as repressor and PtkA as kinase. The kinase PtkA and kinase-activator TkmA expression is induced by the benzoate/xy/s system. The PtkA-TkmA complex can phosphorylate the repressor FatR, which attenuates the DNA binding of FatR to the operator site in front of *gfp*. Derepression of the operator site leads to expression of *gfp* and thus an increase in GFP/mCherry ratio. Different promoter strengths are used to drive the transcription of the *fatR* repressor. mCherry fluorescence is used to normalize the fluorescent signal of GFP.

### 3. Test: using Fluorescence Activated Cell Sorting (FACS)

**Figure 3a:** Raw FACS counts of 5 different strains with various *fatR* (repressor) genotypes under conditions where the kinase operon is not expressed. These strains each display a different *fatR* activity, which is reflected in the signal in the GFP channel (FITC-A). A strong promoter driving *fatR* results in a low GFP signal (green), whereas the strain containing no *fatR* shows a high GFP signal (red). Only one biological replicate of each strain is shown for clarity. This is the data used to calculate the values in Figure 3b.

**Figure 3b:** GFP/mCherry ratio measured of 5 different strains with various *fatR* (repressor) genotypes (based on data from Figure 3a). The two negative control reporters *fatR* Y45E and Δ*fatR* show an expected high ratio of GFP/mCherry. A stronger promoter driving *fatR* transcription results in more repression of GFP and thus a lower GFP/mCherry ratio as expected.

**Figure 3c:** Circuit response of *E. coli* containing both the platform plasmid and the kinase plasmid. Increasing amounts of benzoate induces the expression of the kinase PtkA and activation domain TkmA. Error bars represent SD of 3 biological replicates. d: The fold increase of GFP/mCherry between 0 and 2 mM benzoate. In the negative control strain (Δ*fatR*, red) the fold increase in GFP/mCherry ratio is 2.99±0.05 upon kinase induction. The strains containing a functional *fatR* gene (green shades), (+•) and (+), show a 3.57±0.13 and 4.17±0.09-fold increase in the GFP/mCherry ratio upon kinase induction, as expected. The strain containing (+••) with the strongest promoter driving *fatR* transcription shows only a 2.33±0.04-fold increase in GFP/mCherry ratio by inducing the kinase. Error bars denote SD of 3 biological replicates.

\[1\] Derouiche A. et al. *Nucleic Acids Res* 2013, 41:3971–81

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**Evand@biosustain.dtu.dk**

+45 24 65 31 62

@EricvdHelm